

TARGETING DNA REPAIR IN CANCER: BEYOND PARP INHIBITORS

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ABSTRACT

Germline aberrations in critical DNA repair and DNA-damage response (DDR) genes cause cancer predisposition, while various tumors harbor somatic mutations causing defective DDR/DNA repair. The concept of synthetic lethality can be exploited in such malignancies, as exemplified by approval of poly(ADP-ribose) polymerase inhibitors for treating *BRCA1/2* mutated ovarian cancers (1). Herein, we detail how cellular DDR processes engage various proteins that sense DNA damage, initiate signaling pathways to promote cell cycle checkpoint activation, trigger apoptosis and coordinate DNA repair. We focus on novel therapeutic strategies targeting promising DDR targets and discuss challenges of patient selection and the development of rational drug combinations.

SIGNIFICANCE: Various inhibitors of DDR components are in preclinical and clinical development. A thorough understanding of DDR pathway complexities must now be combined with strategies and lessons learnt from the successful registration of PARP inhibitors in order to fully exploit the potential of DDR inhibitors and to ensure their long-term clinical success.

INTRODUCTION

Failure to accurately repair damaged DNA in cells manifests in various clinical phenotypes, including neurodegeneration, infertility, immunodeficiencies and cancer susceptibility (2). Furthermore, the production of DNA damage in cells following exposure to carcinogens increases cancer risk (3). Germline mutations in genes encoding key players in the DNA damage response (DDR), including *BRCA1*, *BRCA2*, *BLM*, *FANCA*, *TP53*, *RAD51C* and *MSH2*, result in cancer susceptibility syndromes (2), in part because failure to adequately protect the genome against endogenous and exogenous sources of DNA damage results in the accumulation of oncogenic mutations. Genomic instability is therefore a recognised hallmark of cancer (3).

Cancer cells often harbor a reduced repertoire of DNA repair and DNA-damage signalling capabilities compared to normal cells, and in some cases cancers also upregulate mutagenic repair pathways that drive oncogenesis (4). Consequently, cancer cells are often more reliant on a subset of repair pathways and are therefore more susceptible to DDR inhibition than are normal cells that maintain full DNA repair/DDR capacity. Faulty cell cycle checkpoint activation and suboptimal DNA repair capability in cancer cells also results in replication stress and subsequent accumulation of DNA damage in tumors. In addition, cancer cells often have dysfunctional redox homeostasis and therefore rely heavily on mechanisms that repair oxidative DNA damage, as well as on enzymes that counteract the incorporation of oxidised DNA precursors into genomic DNA (5,6). Both replication and oxidative stress, as well as other processes such as telomere attrition, provide

a background of ongoing DNA damage in cancer cells that can provide potential therapeutic windows for compounds that exacerbate these processes. Such compounds may achieve this by stressing replication further, impairing the ability of cancer cells to handle high levels of replicative or oxidative pressures, or potentially inhibiting DNA repair and associated processes (5–7). Such issues have led to intense interest in the therapeutic development of specific inhibitors of a range of components of the DDR network, several of which are now in clinical testing (8).

A well-recognised sensor of DNA damage is the protein poly(ADP-ribose) polymerase (PARP), which is best known for its role in DNA base excision repair (BER) and repair of DNA single-strand breaks (SSBs; **Table 1 + Figure 1**) (9), although it also has a less well-defined role in DNA double-strand break (DSB) repair by alternative non-homologous end-joining (alt-NHEJ; **Table 1 + Figure 1**) (10). The clinical development of PARP inhibitors in patients with germline *BRCA1/2* mutations stemmed from the robust pre-clinical data that demonstrated exquisite sensitivity of *BRCA1/2* mutant cells and tumors to PARP inhibition (11,12). It was correctly hypothesised that in these patients, the cancer cells (in which both alleles of either *BRCA1* or *BRCA2* have been mutated or deleted) would depend on PARP activity for survival, whereas normal cells (that maintain a fully functional copy of *BRCA1* or *BRCA2*) would not. While these findings were initially thought to be due to a reliance of *BRCA1/2* mutant cells on SSB-repair for survival, it has since become well-recognised that PARP trapping and the subsequent generation of replication-dependent DSBs also contributes significantly to the synthetic

lethal relationship between PARP and BRCA. In addition, the PARP family of enzymes plays key roles in multiple cellular processes beyond DNA repair, including cellular differentiation, gene transcription, inflammation, mitosis, cell death and metabolism, which may contribute to the antitumor activity of PARP inhibitors (13,14). Synthetic lethality between repair pathways has provided a paradigm for many current clinical strategies targeting DNA repair/DDR. Detailed reviews of the underlying mechanisms-of-action and clinical applications of PARP inhibitors have previously been published and are beyond the scope of this article (15–19).

In this review, we summarise the current status with PARP inhibitors and then look beyond these, focusing on other protein components of the cellular DDR, which includes proteins that sense DNA damage, initiate signaling pathways that promote cell cycle checkpoint activation and coordinate the repair of damaged DNA with various other cellular processes. We highlight how such DDR enzymes represent rational targets for the discovery of novel therapeutics (**Figure 1**) and detail the development and future potential of such compounds. We also discuss the numerous challenges of discovering predictive biomarkers of response for optimal patient selection and the development of promising DDR combinations, including molecularly-targeted agents, compounds inhibiting epigenetic targets and immune checkpoint inhibitors.

DRUG TARGETING OF DNA DAMAGE SENSOR PROTEINS

DDR sensor proteins detect the region of damaged DNA and direct ensuing cellular responses that include activation of one or more repair pathways. For DSBs, Ku (comprising the Ku70/Ku80 protein heterodimer) and MRN (MRE11-RAD50-NBS1) are the predominant sensor protein complexes. Ku binds DNA DSBs within seconds of them being generated, and serves as a platform for the subsequent recruitment of classical non-homologous end-joining (NHEJ) proteins. The MRN complex plays key roles in triggering activation of the DNA-damage signalling kinase ATM, the initiation of DNA end-resection, and promotion of repair by HR. Chromatin context, transcriptional status, cell cycle stage and extent of end-resection are all factors that contribute to the selection of DNA repair, either by HR or NHEJ, and the mechanisms determining the choice of DNA repair pathway is an intense area of active research (20,21). Other DNA-damage sensors include components of the Fanconi anemia core complex (FANCA, B, C, E, F, G, L and M), mismatch repair proteins (MSH2, MSH3, MSH6, MLH1 and PMS2) and nucleotide excision repair proteins (XPC, DDB2 and CSA), which are sensors of DNA inter-strand crosslinks, base-base mismatches or insertion-deletion loops and UV-induced photo-lesions (in particular cyclobutane pyrimidine dimers and pyrimidine 6-4 pyrimidone photoproducts), respectively **(Table 1)**.

There are 17 PARP family members, of which PARP-1 has the predominant role in DNA repair, with PARP-2 and to a lesser extent PARP-3 functioning in fewer, but overlapping DNA repair processes (22). Through binding to single-

stranded DNA breaks (SSBs), DNA nicks or DSBs, PARP catalytic function is activated to generate extensive poly(ADP-ribose) chains (PAR chains) on itself and proteins in the vicinity of DNA damage. These PAR chains and PARP itself then promote the recruitment of critical SSB repair proteins, such as XRCC1 to SSBs and modify chromatin structure to facilitate DNA repair (9). PARP auto-PARylation is also required for the dissociation of PARP from DNA-damage sites (23,24). Enzymatic inhibition by PARP inhibitors therefore results in both the suppression of SSB repair and BER, which molecularly converges with SSB repair in its downstream stages. Inhibition also results in the trapping of PARP to SSBs, causing the stalling and subsequent collapse of DNA replication forks, resulting in replication-dependent DNA DSBs (23,24). Such DSBs would normally be repaired by HR; however in HR deficient cells, such as *BRCA1/2* mutant tumors, less effective and lower fidelity methods of repair are utilised, which result in an unsustainable levels of damage, chromosomal fusions/translocations and ultimately cell death (20,25).

Following strong preclinical findings predicting a therapeutic rationale, early clinical trials assessing the PARP inhibitor olaparib (Lynparza; AstraZeneca) demonstrated multiple durable antitumor responses in patients with advanced germline *BRCA1/2* mutated ovarian, breast or castration resistant prostate cancer (CRPC) (26,27). This patient benefit was confirmed in later-phase clinical trials (28–31) eventually leading to clinical registration. Olaparib is now approved by the European Medicines Agency (EMA) as maintenance therapy for responding patients with *BRCA1/2* mutant ovarian cancer following platinum-based chemotherapy. It was also granted accelerated approval by

the US Food and Drug Administration (FDA) for use in patients with advanced *BRCA1/2* mutant ovarian cancers, while confirmatory trials are being completed. Most recently, olaparib was given breakthrough therapy designation for treatment of *BRCA1/2* or *ATM* gene mutated metastatic CRPC in patients who have received a prior taxane-based chemotherapy and at least one newer hormonal agent. Another potent PARP inhibitor, rucaparib (Clovis), has also recently been granted breakthrough therapy status by the FDA following the results of the Phase II ARIEL2 trial (32) for use as monotherapy in patients with *BRCA1/2* mutant (germline or somatic) advanced ovarian cancer after at least two prior lines of platinum-containing therapies (33). There are additional potent and selective PARP inhibitors in late phase monotherapy and combination clinical trial development, including niraparib (MK4827; Tesaro), talazoparib (BMN673; Medivation) and veliparib (ABT-888; Abbvie) (**Table 2**).

Although PARP inhibition is undeniably an effective treatment for *BRCA1/2* mutated cancers, with response rates in the region of 50% for platinum-sensitive ovarian cancers (33), the overwhelming majority of patients will ultimately develop tumor resistance. Genetic reversion events that restore *BRCA1/2* gene function have been identified in PARP inhibitor resistant cell lines, platinum-resistant patient-derived cell lines and tumors from patients that have developed clinical resistance to PARP inhibitors (34). In addition, in *Brca1-null* mouse embryonic stem cells, loss of DDR factors such as 53BP1 at least partially rescues HR by removing a barrier to DNA end-resection (35,36), although this has yet to be rigorously identified as a resistance

mechanism in the clinic. A number of critical challenges therefore remain to optimise the clinical efficacy and widen the utility of PARP inhibitors. Identifying mechanisms of PARP inhibitor resistance remains a critical challenge; others include the development of promising PARP inhibitor combination regimens and the analytical validation of clinically meaningful predictive biomarker assays to identify HR-deficient tumors caused by *BRCA1/2* mutations or by other mechanisms (33).

TARGETING OF DNA DAMAGE SIGNALLING PROTEINS

By triggering various protein post-translational modifications and promoting the assembly of protein complexes, DDR signalling proteins amplify and diversify the damage signal within a cell and coordinate the most appropriate cellular responses, including transcriptional changes, cell cycle checkpoint activation, alternative splicing, engagement of DNA repair processes, or in the context of overwhelming damage, activation of cell senescence or apoptotic pathways (2). DNA DSB signalling events are largely coordinated by the apical phosphatidylinositol 3-kinase-related kinases (PIKKs) DNA-PKcs (DNA-dependent serine/threonine protein kinase catalytic subunit), ATM (ataxia telangiectasia mutated), and ATR (ataxia telangiectasia and Rad3-related protein; **Figure 2**).

DNA-PK

DNA-PKcs activity is essential for effective repair by classical NHEJ, which is the predominant DNA repair pathway of DSBs in human cells, occurring through all phases of the cell cycle (**Figure 2A**). DNA-PK is composed of Ku

plus a ~460 kDa catalytic subunit (DNA-PKcs), the activity of which is dependent on Ku-mediated DNA DSB binding (37). Ku binds to DNA DSBs and serves as a platform for the recruitment of other core NHEJ proteins including DNA-PKcs, XRCC4, LIG4, XLF and PAXX amongst others (38,39). Upon DNA binding, autophosphorylation of DNA-PKcs induces a conformational change that destabilises the NHEJ core complex, causing inward sliding of Ku on the DNA and enabling access of end-processing and ligation enzymes to DNA ends to facilitate repair (40). Autophosphorylation also stimulates the dissociation of DNA-PKcs from DNA and Ku, and inactivates the kinase activity of DNA-PK (38). The best described substrate for DNA-PK is itself, with autophosphorylation occurring at multiple sites (40).

As well as being important for the repair of exogenous DNA DSBs, classical NHEJ also plays crucial roles in the repair of endogenous DSBs arising during physiological process, such as V(D)J and class-switch recombination (41). Mice with a disrupted *Prkdc* gene have severe combined immunodeficiency, as well as being radiosensitive (41). In addition, DNA-PK has an established role in innate immunity and pro-inflammatory signalling, which are important issues to consider with respect to long-term treatment with DNA-PK inhibitors, as well as the potential for combination approaches with immune-modulating agents (40). Interestingly, DNA-PK has also long been known to have links to transcription (42), and studies are currently ongoing to investigate the mechanisms by which transcriptional regulation by DNA-PK affects DNA repair (43).

DNA-PK mutants lacking kinase activity, or treatment of cells with small molecule inhibitors of DNA-PK kinase activity cause the latter to be stabilised on DNA ends, impeding NHEJ and also likely interfering with other repair processes, including HR by obstructing DNA end-resection (38). As it plays key roles in repair by NHEJ, DNA-PK inhibition profoundly hypersensitises cells and tumor xenografts to replication-independent DSB-inducing agents, such as radiotherapy and topoisomerase 2 inhibitors (44). In contrast, DNA-PK inhibition alone has very little effect on cancer cell or tumor viability (44), perhaps because most endogenous DSBs arise in the context of DNA replication, where the preferred repair pathway is HR. These compounds are therefore predicted to be associated with modest antitumor activity as monotherapy, while, there is potential for antitumor synergy in combination with DNA damaging agents, albeit with a potentially narrow therapeutic index because of associated effects on normal cells in the patient.

The clinical development of DNA-PK inhibitors with high potency and selectivity *in vitro* has been complicated by inadequate pharmacokinetic (PK) properties. However, a number of novel DNA-PK inhibitors have recently entered clinical development (**Table 2**). For instance, MSC2490484A (Merck KGaA; NCT02316197) is being evaluated in phase I trials as monotherapy and in combination with radiotherapy, while a phase I trial of VX-984 (Vertex pharmaceuticals) in combination with liposomal doxorubicin has recently started recruitment (NCT02644278). A dual inhibitor of DNA-PK and TOR kinase (a downstream effector of the PI3K-AKT pathway signaling and another member of the PIKK family), is CC-115 (Celgene), which was

developed through lead optimisation of existing mTOR inhibitors (45). In pre-clinical studies, CC-115 inhibits proliferation and induces caspase-dependant cell death in chronic lymphoid leukaemia (CLL) cells, and also leads to death of CLL cells resistant to the PI3K δ inhibitor idelalisib (46). The relative importance of DNA-PK versus TOR inhibition in this setting has however not been fully elucidated. In a recently reported phase I trial involving patients with advanced solid and hematological malignancies, CC-115 was well tolerated, with preliminary antitumor activity observed (47). There has been some suggestion that CC-115 may have greater activity in patients with CLL harboring biallelic *ATM* loss, although the mechanism for this has yet to be established (46).

ATM

Similar to DNA-PK, ATM promotes DNA DSB repair in cells and responds to DSBs generated throughout the cell cycle. Inherited mutations in the *ATM* gene result in the autosomal recessive condition Ataxia Telangiectasia, a syndrome characterised by progressive cerebellar ataxia, oculocutaneous telangiectasia, radiosensitivity, predisposition to lymphoid malignancies and immunodeficiency, with defects in both cellular and humoral immunity (48). A number of different factors have now been identified that promote ATM activation; however, following DNA DSBs, ATM is predominantly activated through interactions with NBS1 of the MRN complex (**Figure 2B**; (49,50). ATM is the principal kinase responsible for the phosphorylation of histone H2AX on serine 139 (known as γ H2AX) (51), although some functional redundancy exists with ATR and DNA-PK. MDC1 (mediator of DNA damage

checkpoint protein-1) binds directly to γ H2AX (52) and potentiates the DNA damage signal, leading to the spreading of γ H2AX to over a megabase from its initial lesion (53). This amplification is thought to help sustain the DDR signal to enable sufficient recruitment and retention of DNA damage mediator proteins such as 53BP1 at sites of DNA damage, which can be visualised as foci in DNA damaged cells.

Phospho-proteomic studies have identified hundreds of ATM substrates (54), although the physiological relevance of many of these proteins is currently unknown. A well-recognised substrate of ATM is CHK2, the activity of which is predominantly, but not exclusively important for G1-S phase checkpoint activation (55). ATM is also important for the stabilisation of p53 through the phosphorylation and subsequent inhibition of proteosomal degradation by MDM2 (55).

ATM inhibition has been demonstrated to hypersensitize cells to ionizing radiation and the DNA DSB-inducing agents etoposide, camptothecin and doxorubicin (56). A phase I trial of the ATM inhibitor AZD0156 (AstraZeneca) is currently underway as monotherapy and in combination with olaparib and other cytotoxic or molecularly targeted agents (NCT02588105; **Table 2**). While there are likely to be other ATM inhibitors in development, to our knowledge, none have reached clinical studies.

ATR

Although also important for DSB repair, the context for ATR activation is different to ATM and DNA-PK. ATR is activated by RPA (replication protein A) bound ssDNA, which can arise as a result of stalled replication forks and also occurs following DNA end-resection during the early stages of homologous recombination (**Figure 2C**) (57). ATR is recruited to RPA-ssDNA by its obligate binding partner ATRIP (ATR-interacting protein), and is activated by TOPBP1 (topoisomerase binding partner 1) in complex with the Rad17-Rfc2-5 clamp loader, the 9-1-1 complex (Rad9-Rad1-Hus1), Claspin and RHINO (58). CHK1 is the best described substrate of ATR and once activated by ATR, CHK1 serves to inhibit cyclin-dependent kinase (CDK) activity through the phosphorylation of CDC25A. As such, CHK1 is a critical regulator of the G2-M and intra-S cell cycle checkpoints (**Figure 2C**) (59). Interestingly, recent preclinical studies have demonstrated that both ATR and CHK1 have distinct roles in the regulation of the intra-S checkpoint. ATR appears particularly important for the suppression of replication catastrophe in early S-phase cells through the promotion of ribonucleotide reductase accumulation and by limiting origin firing (60). In contrast, other S-phase cells are capable of recovering from replication insults through a CHK1-mediated back-up mechanism (60). A synthetic lethal relationship has now been established between ATR and CHK1 inhibition, with combination blockade leading to replication fork arrest, DNA SSB accumulation, replication collapse and synergistic cell death in cancer cells *in vitro* and *in vivo* (61).

In addition, a third checkpoint kinase has now been identified (MK2; MAPKAP-K2), which functions independently of CHK1, downstream of ATM

and ATR to maintain G2/M and intra-S phase arrest (62–64). To our knowledge, there are currently no MK2 inhibitors in clinical development, although pre-clinical work has demonstrated interesting synergy between MK2 and CHK1 inhibitors, particularly in *KRAS* mutant tumors (65).

VX-970 (Vertex pharmaceuticals) is a first-in-class ATR inhibitor, with preclinical data demonstrating chemosensitization of lung cancer cells predominantly to chemotherapeutics that result in replication fork collapse, such as cisplatin and gemcitabine *in vitro*, and increased antitumor activity in combination with cisplatin *in vivo* (66,67). Preliminary phase I trial data have shown that VX-970 is well tolerated as monotherapy with no dose limiting toxicities or grade 3-4 adverse events demonstrated up to weekly intravenous (IV) doses of 480mg/m² (68). A durable RECIST (response evaluation criteria in solid tumors) complete response was observed in a patient with metastatic ATM-loss colorectal cancer, who remained on single agent VX-970 for more than 20 months. When VX-970 was combined with carboplatin, although the maximum tolerated dose was not established, because there was no more than 1 dose-limiting toxicity at each dose level, the recommended phase II dose (RP2D) was VX-970 90 mg/m² + carboplatin area under the curve (AUC) 5 based on carboplatin dose delays observed at higher dose levels. Crucially, paired tumor biopsy studies undertaken at this RP2D showed significant inhibition of ATR-targeted phosphorylation of Ser-345 on CHK1, confirming target modulation. At the RP2D, a patient with platinum-refractory, PARP inhibitor resistant, germline *BRCA1* and *TP53* mutant advanced high-grade serous ovarian cancer achieved a RECIST partial response and gynecologic

cancer intergroup (GCIG) CA125 tumor marker response lasting 6 months. As expected from the predicted mechanism-based toxicity profile of an ATR inhibitor and platinum chemotherapy, myelosuppression (neutropenia and thrombocytopenia) was the most commonly observed treatment-related toxicity (69). Combination trials with VX-970 and a number of other chemotherapeutics are ongoing, including cisplatin and gemcitabine, with promising antitumor responses observed in chemotherapy-resistant patients with advanced solid cancers (70,71) (**Table 2**). AZD6738 (AstraZeneca) is an oral ATR inhibitor currently being assessed in phase I clinical trials as monotherapy or in combination regimens with olaparib, carboplatin, radiotherapy or the immune-checkpoint inhibitor durvalumab (MEDI4736; AstraZeneca). The optimal scheduling and sequencing of these agents with their respective partners, in order to balance the trade-off between antitumor activity and bone marrow toxicity, is not yet clear and full results of these trials are awaited with interest.

CHK1

Pre-clinically, CHK1 inhibitors have demonstrated most synergy with drugs that generate replication dependent DNA damage such as anti-metabolites, and therefore clinical development has focused on their use in combination with such drugs (72). MK8776 (Merck & Co) is a potent and selective CHK1 inhibitor that is well tolerated as a monotherapy, as well as in combination with gemcitabine (**Table 2**) (73). Results from a recently published phase I trial of MK8776 as monotherapy or in combination with gemcitabine, has shown preliminary evidence of clinical efficacy, with 2/30 patients (7%) having

a partial response and 13/30 (43%) demonstrating stable disease (73). As expected, toxicity was more frequent in combination, and included fatigue, nausea, anorexia, thrombocytopenia, neutropenia and transient, dose-related electrocardiogram (ECG) abnormalities, specifically QTc prolongation. The recommended phase II dose of MK8776 is 200mg, with gemcitabine administered at 1000 mg/m² on days 1 and 8 of a 21-day cycle. LY2603618 (Eli Lilly) is a selective CHK1 inhibitor being evaluated at both 170mg and 230mg in combination with gemcitabine (**Table 2**) (74). Preliminary results reported RECIST partial responses in 4/17 patients, with mainly hematological toxicities observed, and three patients discontinuing treatment because of adverse events. A phase I trial of CHK1 inhibitor CCT245737 (Sareum Holdings plc) as monotherapy, and in combination with cisplatin and gemcitabine has recently started accrual (NCT02797977, NCT02797964) (**Table 2**).

CHK2

There is some uncertainty as to whether the inhibition of CHK2 will be beneficial in the clinical setting, and at present there are no selective CHK2 inhibitors in clinical development (72). Genetic deletion of the mouse *Chk2* gene alleviates p53-dependent cell death following irradiation and although the mechanisms for this protection have not been fully defined, there is a hypothetical risk that inhibition of CHK2 may be radio-protective in a clinical setting (75). Further studies are required to determine the appropriate clinical context in which CHK2 inhibition may lead to antitumor activity (76). Several of the early cell cycle checkpoint inhibitors, such as LY2606368 (Prexasertib;

Eli Lilly), are dual inhibitors of CHK1 and CHK2, and many of these have now discontinued clinical development due to lack of efficacy. LY2606368 has undergone evaluation in a phase I trial, defining a RP2D of 105mg/m² every 14 days, with a predominant toxicity of myelosuppression (**Table 2**) (77). Evidence of single agent activity was observed, with 2/45 patients achieving a RECIST partial response (one with anal cancer and one with head and neck squamous cell carcinoma), while 15/45 (33.3%) patients obtained clinical benefit with radiological stable disease. The trial is expanding, preferentially in patients with squamous histology tumors, and several combination strategies are currently ongoing.

WEE1

Working in parallel with CHK1, the WEE1 protein kinase also plays a critical role in the activation of the G2-M checkpoint through the regulation of cyclin dependent kinases (78,79). Unlike CHK1, however, WEE1 is not directly regulated by DNA damage, but is required for physiological cell cycle progression. The predominant mechanism-of-action of WEE1 inhibitors was initially believed to be failure of the G2-M checkpoint through inhibition of CDK1 Tyr15 phosphorylation, inactivation of the CDK1/CCNB1 complex and subsequent mitotic catastrophe (80). More recently, however, it has become clear that WEE1 inhibition also generates replication-dependent DNA damage in cells, due to aberrant DNA replication and CDK2 inhibition (81,82). The first-in-class WEE1 kinase inhibitor AZD1775 (MK1775; AstraZeneca) has been shown to potentiate the cytotoxic effects of a range of DNA damaging agents and demonstrate single agent activity In preclinical models (78).

AZD1775 has been evaluated in a single agent phase I clinical trial (83), where a maximum tolerated dose of 225mg twice daily for 2.5 days per week for two weeks in three-weekly cycles was established (**Table 2**). Dose limiting toxicities reported were reversible supraventricular tachycardia and myelosuppression, with common toxicities including myelosuppression and diarrhea. The study noted evidence of single agent activity with RECIST partial responses in two germline *BRCA1* mutant patients (papillary serous ovarian and squamous cell carcinoma of the head and neck). Proof-of-mechanism target modulation was demonstrated in paired tumor biopsies demonstrating reduced CDK1 Tyr15 phospho levels and increased γ H2AX levels after treatment.

Preliminary data from a phase II trial of AZD1775 in combination with carboplatin and paclitaxel versus chemotherapy alone in patients with platinum-sensitive TP53-mutant ovarian cancer demonstrated a superior progression free survival (PFS) benefit [hazard ratio (HR) 0.55, CI 0.32-0.95, $p=0.030$], with common toxicities including nausea, diarrhea, alopecia and fatigue (**Table 2**) (84). Interestingly, there is also evidence that WEE1 inhibition may reverse platinum resistance, as the combination of AZD1775 and carboplatin has shown antitumor activity in patients with *TP53* mutant, platinum resistant/refractory ovarian cancer, with a reported RECIST partial response rate of 27% in evaluable patients to date (**Table 2**) (85). There is now a need to better define the patient populations predicted to respond to AZD1775 monotherapy and novel combination regimens, and numerous

biomarker-driven clinical trials with AZD1775 are currently ongoing to address such issues (**Table 2**).

TARGETING DDR EFFECTOR PROTEINS AND REPAIR PATHWAYS

DDR events converge on one or more repair pathways that are dedicated to specific types of DNA damage (**Figure 1 and Table 1**). Some established antitumor agents result in a single type of DNA lesion (e.g. topoisomerase inhibitors), while others generate a heterogeneous mixture of DNA damage types, engaging multiple repair pathways simultaneously (e.g. radiotherapy). While NHEJ and HR remain the predominant DSB repair pathways, the importance of alternative homology-directed repair mechanisms is also now recognised (20). These are mutagenic pathways that are able to 'back-up' standard repair processes, which have either been genetically or chemically compromised. While there are multiple attractive DNA repair and DDR effector protein targets, there are still only a limited number of drugs currently in clinical development that target such proteins.

APE1 (AP endonuclease-1) recognises abasic (AP) sites generated following the removal of damaged bases by DNA glycosylases, and its endonuclease activity is essential for BER (86). In addition, APE1 harbors exonuclease activity important for the removal of 3' obstructive lesions in DNA, including chain-terminating nucleoside analogs (87). TRC102 (Methoxyamine; TRACON pharmaceuticals) reacts with abasic sites to cause an AP-adduct that is resistant to APE1 action (88), exacerbating the cytotoxicity of alkylating agents and anti-metabolites in cells. Hematological toxicities were dose

limiting for TRC102 in combination with pemetrexed or temozolomide (89,90), and other early phase combination trials are ongoing (**Table 2**).

Recent studies have demonstrated that HR deficient cells rely on error-prone microhomology-mediated end-joining (MMEJ; also known as alt-NHEJ) for survival (91,92). The polymerase activity of POLQ (DNA polymerase theta) is required for gap-filling during MMEJ, and POLQ also prevents hyper-recombination by limiting RAD51 accumulation at resected DNA ends (91,92). POLQ is therefore an attractive drug target, particularly in the context of HR deficient tumors. The development of small molecule inhibitors that target protein-protein interactions of the RAD51 recombinase family are also ongoing (93). Targeting protein-protein interactions is challenging and has had limited success, although compounds have been identified that disrupt the self-association of RAD51 and successfully inhibit the interaction between RAD51 and BRCA1 (93). These compounds have the potential to inhibit RAD51-dependent HR in cells, and the demonstration of such effects in functional cellular assays is awaited.

FINE-TUNING THE DDR

Each step of the DDR is tightly regulated by reversible post-translational modifications (PTMs) including: phosphorylation, ADP-ribosylation, methylation, acetylation, ubiquitylation, sumoylation and neddylation (94–97). While DDR-specific kinase and ADP-ribosylation inhibitors are already in clinical development/use, given the essential role of ubiquitylation and de-ubiquitylation in the DDR, modulating DNA repair through the use of specific

inhibitors of ubiquitylation, deubiquitylation or the ubiquitin-proteasome system is an active area of research (97,98). HR is particularly sensitive to proteasome inhibition (99) and proteasome inhibitors such as bortezomib (Velcade; Takeda Pharmaceutical Company Ltd) have been shown to block global ubiquitylation in cells, and disrupt protein turnover of several DDR proteins, such as MDC1, BRCA1 and RPA (100–102). Inhibiting a subset of ubiquitin ligases, namely the cullin-ring-ligases (CRLs), through inhibition of neddylation (the covalent attachment of the ubiquitin-like protein, NEDD8 to target proteins) in cells, also affects the DDR (94,103). Pevonedistat (MLN4924; Takeda Pharmaceutical Company Ltd) inhibits the NEDD8 E1, blocking NEDD8 conjugation and CRL activity in cells (104). A phase I study of pevonedistat showed that an intermittent dosing schedule was generally well tolerated, with hepatotoxicity being dose-limiting (105). In pre-clinical studies, pevonedistat exhibited particular synergy with DNA cross-linking agents (106,107) and phase I combination studies are currently ongoing.

PTMs are reversible, with such turnover being important for various cellular processes. It's perhaps unsurprising therefore that deubiquitylating enzymes (DUBs) also play key roles in promoting DNA repair in cells. Indeed, various DUBs are attractive drug targets (97), with several DUB inhibitors currently in pre-clinical development. Analogously, poly(ADP-ribose) glycohydrolase (PARG) catalyses the hydrolysis of poly(ADP-ribose) and therefore reverses the effects of PARP. Inhibition of PARG, in a similar fashion to PARP inhibition, leads to DNA damage that depends on HR for repair (108), and efforts are ongoing to generate specific PARG inhibitors for clinical use (109).

Several other classes of compounds have demonstrated inhibitory effects on the DDR, which may potentially be exploited in a clinical setting. Chromatin compaction significantly affects DNA repair (21) and the chromatin modifying inhibitors vorinostat (ZolanzaTM; Merck) and romidepsin (Istodax; Celgene) are both approved for the treatment of cutaneous T cell lymphoma (110). As well as relieving chromatin compaction, these histone deacetylase (HDAC) inhibitors also transcriptionally down-regulate a number of DSB repair proteins, thereby hypersensitising cells to DSB-inducing agents and providing strong rationale for combination treatment with DNA-damaging compounds (111,112). There are numerous other compounds in pre-clinical and clinical development, which have inhibitory effects on DNA repair through targeting epigenetic modifier enzymes, such as EZH2 (H3K27 methyltransferase) (113), histone deacetylases (HDACs) (112) and G9A (histone lysine N-methyltransferase) (114).

PATIENT SELECTION

PARP inhibitors are selectively toxic to tumor cells with biallelic mutations/loss of *BRCA1* or *BRCA2* (*BRCA1/2*) (11,12), and olaparib is the first oncology drug to be licensed with a companion genetic diagnostic (BRCAanalysis CDxTM). Such a level of “synthetic lethality” has yet to be reproduced with any other DDR inhibitor. The effectiveness of PARP inhibitors is not restricted to patients with germline or somatic *BRCA1/2* mutations however, and significant efforts are underway to determine tumors that are essentially HR deficient through other mechanisms (115). Genomic approaches to achieve

this include studies undertaken to identify mutations in single HR and/or other genes that predict for PARP inhibitor sensitivity (116,117). In addition, DNA repair dysfunction has the potential to lead to global DNA aberrations; the presence of a genome-wide mutational signature (or genomic scar) that occurs in the context of chronic HR deficiency, may thus also be a useful biomarker that is predictive of PARP inhibitor sensitivity (118). Scoring systems that measure genomic defects reflective of HR deficiency are also being utilised, including those that quantify loss of heterozygosity (LOH), telomeric allelic imbalance and large scale state transitions (defined as a chromosomal break between adjacent segments of DNA of at least 10 Mb) within tumors (119).

The hypermethylation of genes and other epigenetic effects mean that focusing entirely on genomics will ultimately fail to identify all patients who are likely to benefit from a molecularly-targeted cancer therapy. To address this issue, functional assays of HR deficiency have been pursued and have included the detection of RAD51 foci at DNA-damage sites in breast cancer biopsies following neo-adjuvant chemotherapy, where a failure to generate RAD51 foci in cells was strongly predictive of a pathological complete response ($p=0.011$) (120). To date, however, there is no robust strategy to clinically determine which patients will benefit from PARP inhibitors outside of the *BRCA1/2* mutant population and it may be that a combination of functional and genomic approaches is required.

Beyond PARP inhibitors, preclinical data have demonstrated synergy between ATR inhibition and impaired ATM signalling, particularly in the context of exogenous DNA damage (121). The mechanism for this synergy has yet to be defined; however, clinical studies exploring ATR inhibition in the context of ATM loss are ongoing (69). Certainly, a number of studies suggest that high levels of replication stress and consequently, increased endogenous DNA damage in tumors may be required for hypersensitivity to ATR inhibitor monotherapy. Overexpression of oncogenes such as *CCNE1*, *CCND2* and *MYC*, adversely affects DNA replication by disrupting origin firing and replication progression, resulting in oncogene-induced replication stress (122). In keeping with this, *CCNE1* amplification exaggerates the hypersensitivity of *TP53* deficient cells to ATR inhibition (123), and both ATR and CHK1 inhibitors are particularly toxic for *Myc*-driven lymphomas in mice (124). In addition, oncogenic stress as a result of activating *KRAS* mutations has been shown to hypersensitise cells to ATR inhibition (125), and selecting tumors with oncogene-induced replication stress has the potential to provide a much-needed therapeutic window for ATR inhibitor/chemotherapy combination strategies.

The physical ends of linear chromosomes, telomere ends, are naturally occurring DNA DSBs in cells that are protected by the Shelterin protein complex in order to prevent DDR activation (126). Maintaining telomere length is essential for the genomic stability of replicating cells and involves the concerted actions of several key DDR players, including ATM, ATR, DNA-PK and Ku (127,128). Telomere maintenance is achieved through telomerase

activation in 85% of cancers and telomerase recruitment to telomeres is dependent on ATM and ATR activity in human cells (128). ATR activity has also been shown to be important for alternative lengthening of telomeres (ALT) (129), a mechanism which relies on recombination events to maintain telomere length. In keeping with this, preclinical data have been published demonstrating hypersensitivity of ALT tumor cells to ATR inhibition (129). Given the role of the DDR PIKKs in telomere maintenance, DNA-PK, ATM and ATR inhibitors, all have the potential to negatively affect telomere length in cells. While this may potentially contribute to their antitumor effects, it may also result in detrimental genomic instability in replicating non-cancer cell populations.

It is currently too preliminary to establish if *TP53* deficiency, ATM loss/mutation, ALT reliance or *CCNE1*, *CCND2* or *MYC* oncogene activation will predict for sensitivity to ATR inhibitors in the clinic, but it is likely that this will be a far from exhaustive list of putative genomic predictive biomarkers, and having a functional marker of replication stress in tumors will be helpful. Surrogate markers of replication stress might include phosphorylation of ATR substrates (e.g. CHK1pS345 and RPApS33) or levels of single-stranded DNA (60). How these markers might change over time and with treatment is of course currently unknown however, and the usefulness of measuring these biomarkers in archival tumor specimens has yet to be tested.

COMBINATION STRATEGIES

Combining DDR inhibitors with DNA damaging agents has been the natural first step in the clinical development of combination strategies for DDR inhibitors (**Table 2**). A thorough understanding of the DNA lesions induced by different chemotherapies, and inhibition of the respective pathways required for repair will ultimately maximise the odds of synergistic antitumor efficacy. Nevertheless, toxicities will in many cases likely limit drug doses used in such combinations. Indeed, combining olaparib with carboplatin and paclitaxel chemotherapies in the clinic has been challenging because of myelosuppression, and reductions in the full single-agent doses of all drugs had to be undertaken to enable the combination to be administered safely (130,131). While olaparib showed promising data in a phase II trial of patients with advanced gastric cancer harboring ATM loss when combined with the paclitaxel chemotherapy (132), there was no statistically significant survival benefit in the phase III GOLD trial (according to a May 18th, 2016 AstraZeneca press release). Optimizing drug scheduling may enable potential differences in repair kinetics of normal versus cancer cells to be exploited and may therefore, increase damage in tumors while sparing normal tissue. Careful consideration of the sequence of combination drug administration is required to optimize synergistic effects. Equally, selecting patients with tumors of specific genotypes or phenotypes may produce a therapeutic window for such combinations.

Combining DDR inhibitors with small molecule inhibitors of other cellular signalling pathways also shows promise, and PARP inhibition has been tested

in combination with a number of agents. For example, an EGFR inhibitor combination has been explored following clinical data from the EURTAC trial showing that low BRCA1 mRNA levels were associated with longer PFS to erlotinib (Tarceva; Genentech) (133). A phase I trial of olaparib with gefitinib (Iressa; AstraZeneca) demonstrated safety and tolerability, as well as promising signals of antitumor activity (134); a phase 2 trial is now accruing (135). Recent pre-clinical data also suggest that inhibition of the receptor tyrosine kinase c-MET hypersensitises cells to PARP inhibition and the clinical evaluation of this finding is likely to follow (136). Preclinical evidence of phosphatidylinositol 3-kinase (PI3K) inhibition impairing BRCA1/2 expression and sensitising tumor cells to PARP inhibition in both *BRCA1/2*-mutant and *BRCA1/2*-wild type breast cancers (137,138) has led to phase I combination trials of olaparib with the PI3K inhibitor BKM120 (Buparlisib; Novartis) (139) and the AKT inhibitor AZD5363 (AstraZeneca) (140), respectively. Preliminary data suggest that these combinations are tolerable and effective, with final results awaited with interest.

Preclinical evidence suggests that hypoxia results in impaired HR through the down-regulation of HR-related genes (141–143). This provided the rationale for a phase 1/2 trial of the pan-vascular endothelial growth factor (VEGF)1-3 inhibitor cediranib (AZD2171; AstraZeneca) with olaparib in patients with platinum-sensitive ovarian cancer (144). A PFS benefit of 8.7 months (HR 0.42 [95% CI 0.23–0.76; $p=0.005$]) was demonstrated with the combination versus olaparib alone in the overall patient population, with predominant toxicities of fatigue, diarrhea and hypertension in the combination arm

observed. Interestingly, however, no PFS difference was observed between the two treatment arms in patients with *BRCA1/2* mutated ovarian cancer.

Apart from molecularly targeted agents, we are also beginning to appreciate the considerable crosstalk between DNA repair and endocrine signalling (145). Steroid hormone signaling has been shown to promote NHEJ through transcriptional regulation of NHEJ components such as *PRKDC*, and has been demonstrated to have both positive and negative effects on HR depending on tumor model and context (145). In prostate cancer models, PARP1 has been demonstrated to support androgen transcriptional function, and is required for transcriptional activation of the oncogenic fusion TMPRSS2-ERG protein found in >50% of prostate cancers. Dual blockade of PARP activity and androgen receptor signaling delays tumor growth compared to either as monotherapy in mouse xenograft prostate cancer models (146). This has led to trials combining PARP inhibitors with hormonal manipulation, such as olaparib with the CYP17 inhibitor abiraterone (Zytiga; Janssen Biotech) (147).

There are also now multiple combination studies involving immune checkpoint inhibitors with DDR inhibitors, such as PARP and ATR inhibitors (**Table 2**). There is pre-clinical evidence to suggest that immune checkpoint inhibition synergizes with PARP inhibitor treatment in BRCA1 deficient tumors and clinical trials investigating this hypothesis are ongoing (148). In addition, the success of anti-PD-1/PD-L1 therapeutics in MMR deficient tumors (149) raises the intriguing question as to whether increasing mutational load with

DDR inhibitors might increase the immunogenicity of cancers and subsequent responses to immunotherapy. Further studies are required to substantiate this hypothesis, and while high levels of microsatellite instability might prove to be a useful biomarker of response to immune checkpoint inhibitors, alternative mechanisms that might be driving sensitivity should not be discounted (150). Equally, we must be mindful that an intact DDR plays an important role in innate immunity (151). DDR signalling is important for the activation of inflammatory cytokines and induces the expression of immune-receptor ligands on damaged cells. As such, inhibitors of DDR signalling may in fact attenuate the immune response following DNA damage and therefore immunotherapy-DDR inhibitor combination studies need to be carefully considered.

FUTURE PERSPECTIVES AND CONCLUSIONS

With multiple DDR inhibitors now in preclinical and clinical pipelines, careful consideration of their mechanisms-of-action is required in order to maximise their potential. DDR-deficient tumors should not be grouped indiscriminately into a class of tumors that may respond to any DDR inhibitor. Through closer collaborations between scientists and clinicians, we must insist on a rational rather than empirical approach to the clinical development of DDR inhibitors. A number of factors will be critical to ensure clinical success, including the development of analytically validated pharmacodynamic assays and predictive biomarkers of response and resistance. As we have observed with PARP inhibitors, managing the toxicities of DDR inhibitor/DNA-damaging agent combinations is likely to be challenging, and so clinicians should not

shy away from aiming for a single agent synthetic lethal approach that has already led to some success in the clinic. Much attention has focused on genetic alterations to key DDR drivers, but the relative contribution of somatic epigenetic loss of such DDR players has not been extensively explored. For example, the silencing of the *BRCA1* gene through promoter hypermethylation has been demonstrated in breast and ovarian cancers (152), which highlights the importance of also considering functional biomarker assays, rather than relying on genomics in isolation.

Modern clinical trial designs will need to incorporate translational studies, which may be used to guide patient selection, drug scheduling and treatment response (153). Early phase trials should aim to consolidate preclinical understandings of drug mechanisms-of-action. Notably, understanding how many successful drugs function, including the PARP inhibitors, has changed over time, meaning that compounds showing preclinical promise should not be discounted on the basis of hypotheses that later appear to be incorrect. It is likely that combination regimens, either with drugs given together or sequentially to overcome resistance, will be required for the optimal application of these DDR inhibitors in the clinic. The use of longitudinal genomic profiling of circulating free DNA to support adaptive drug administration will also be important.

The long-term effects of inhibiting the DDR in patients are still not known and needs further study. One recognised risk of DNA damage to normal tissue is the emergence of secondary cancers, particularly hematological malignancies,

following chemotherapy treatment and the potential mutagenic effects of inhibiting DNA repair. We will need to increase our clinical experience of DDR inhibitors before the long-term effects of these compounds are realised. Nevertheless, as PARP inhibitors move into the neo-adjuvant and adjuvant settings, the malignant potential of these drugs must be monitored.

Precision medicine has heralded the advent of sophisticated modern technologies, which have permitted genomic profiling of both normal and tumor tissue at greater speeds and at lower costs than before. This has enabled the “real-time” identification of germline and somatic DNA repair gene aberrations, which has critical implications both for identifying families at risk of cancer predisposition, and also for predicting therapeutic responses to DDR inhibitors. Now that olaparib has been approved for clinical use and others will hopefully soon follow, we must not forget the lessons learned from the successful development of PARP inhibitors, nor ignore the multitude of opportunities that still exist within the DDR network, which now need to be exploited to impact positively on cancer medicine.

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Double strand break repair pathways	
Classical (c)-NHEJ	<ul style="list-style-type: none"> • Predominant DNA DSB repair pathway in human cells, functioning throughout the cell cycle. • Involves the relatively rapid ligation of broken DNA ends, mediated by the core NHEJ complex including, DNA-PK, XRCC4, LIG4, XLF and PAXX amongst others. • DNA end-processing and DNA polymerase action may be required before ligation can occur, making NHEJ inherently error-prone. • NHEJ maintains genome stability however, by rapidly repairing DSBs in circumstances where recombinogenic events would likely result in gross chromosomal rearrangements; in non-cycling or G1 cells for example (38,39).
Homology-directed repair	
Homologous recombination (HR)	<ul style="list-style-type: none"> • Relatively slow and restricted to late-S phase/G2 as it generally relies on a homologous sister chromatid DNA strand for repair. • Extensive DNA end-resection by helicases and exonucleases such as DNA2, BLM, WRN and EXO1 results in a 3' –ssDNA overhang, committing the break to repair by HR. • RPA coats and stabilizes the ssDNA, leading to ATR activation and subsequent signaling events. • BRCA2, with the help of BRCA1 and PALB2, load RAD51 onto the RPA-coated ssDNA leading to strand invasion, with a number of factors negatively regulating this process to prevent hyper-recombination such as POLQ, PARI, RECQL5, FANCI and BLM (154).
Alternative (Alt)- NHEJ or microhomology mediated end-joining (MMEJ)	<ul style="list-style-type: none"> • Ligation pathway for DSBs when c-NHEJ is genetically compromised (155). • Occurs following limited DNA end resection. • Contributes to the excessive genomic deletions and chromosomal translocations seen in tumors and may also provide a back-up repair pathway in HR deficient cells (10,20).
Single-strand annealing (SSA)	<ul style="list-style-type: none"> • Mutagenic, RAD51 independent repair pathway, involving annealing of short or longer complementary DNA sequences on resected DNA with subsequent deletion of the intervening DNA sequence. The detailed mechanism has yet to be defined in mammalian cells (20).
Other repair pathways	
Inter-strand crosslink (ICL) repair	<ul style="list-style-type: none"> • ICLs cause DNA replication fork stalling and collapse, resulting in DNA DSBs. • ICLs are recognised by the FANCONI core complex, which engages HR, TLS and NER pathways to repair the DNA lesion (156).
Single-strand break (SSB) repair	<ul style="list-style-type: none"> • SSBs usually arise following the removal of a damaged nucleotide (157). • PARP1 (poly-ADP-ribose polymerase 1) is the DNA damage sensor protein for DNA strand breaks. PARP1 localises to sites of DNA damage, generating extensive PAR (poly ADP-ribose) chains. • Ribosylated PARP1 promotes recruitment of SSB-repair proteins to DNA damage sites (9).
Base excision repair (BER)	<ul style="list-style-type: none"> • DNA glycosylases recognize and remove damaged bases leading to basic sites that are processed by APE1 (AP-endonuclease 1). • Results in SSB generation, repaired using SSB repair pathways (86).
Tran-lesion synthesis (TLS)	<ul style="list-style-type: none"> • DNA damage tolerance pathway that helps prevent replication fork stalling (158). • Engages low-fidelity DNA Y-family polymerases (e.g. REV1, POLH, POLI, POLK) that accommodate the damaged lesion, replicating past it, at the expense of increased mutagenesis.
Nucleotide excision repair (NER)	<ul style="list-style-type: none"> • Removes helix-distorting lesions from DNA, in particular the UV-induced photo lesions. • Involves removal of a short oligonucleotide including the damaged lesion using structure specific endonucleases and subsequent restoration of the DNA sequence by DNA polymerases (159).

Mismatch repair (MMR)	<ul style="list-style-type: none"> MSH2, MSH3 and MSH6 recognize base-base mismatches and IDLs, where they recruit MLH1 and PMS2 to damaged sites. The concerted actions of the mismatch repair proteins, engage EXO1 to remove the mismatch and then POLD and LIG1 to fill the gap and seal the nick respectively (160).
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Table 1: Predominant DNA repair pathways.

Target	Agent	Phase	Intervention	Cancer(s) enrolled	Status / results	Trial identifier	Ref.
DNA-PK	MSC2490484A	I	MSC2490484A	Solid tumors, CLL	Recruiting	NCT02316197	-
	MSC2490484A	I	MSC2490484A ± RT	Solid tumors	Recruiting	NCT02516813	-
	VX-984	I	VX-984 ± PLD	Solid tumors	Recruiting	NCT02644278	-
	CC-115 ¹	I	CC-115	GBM, HNSCC, Prostate, ES, CLL	37% (3/8) PR in relapsed ATM ^{mut} CLL	NCT01353625	(46,47)
ATM	AZD0156	I	AZD0156 ± olaparib	Solid tumors	Recruiting	NCT02588105	-
ATR	VX-970 ± chemotherapy	I	VX-970 ± carboplatin	Solid tumors	Recruiting	EudraCT: 2013-005100-34	(68,69)
		I	VX-970 ± gemcitabine, etoposide, cisplatin or carboplatin	Solid tumors	Recruiting	NCT02157792	(70,71)
		II	Gemcitabine ± VX-970 (randomised)	Ovarian, primary peritoneal or Fallopian tube	Recruiting	NCT02595892	-
		I	VX-970 + irinotecan	Solid tumors	Recruiting	NCT02595931	-
		II	Carboplatin + gemcitabine ± VX-970 (randomised)	Advanced gynecologic cancers	Not yet recruiting	NCT02627443	-
		I / II	VX-970 + topotecan	Advanced NSCLC, SCLC, Gynae or neuroendocrine	Recruiting	NCT02487095	-
		II	Cisplatin + gemcitabine ± VX-970 (randomised)	Advanced urothelial	Recruiting	NCT02567409	-
	VX-970 ± RT	I	Cisplatin + RT ± VX-970	Locally advanced HNSCC	Recruiting	NCT02567422	-
		I	Whole brain RT + VX-970	NSCLC brain metastases	Recruiting	NCT02589522	-

	VX-970 + targeted therapy	I	VX-970 + veliparib + cisplatin	Solid tumors	Recruiting	NCT02723864	-
	AZD6738	I	AZD6738	Relapsed CLL, PLL, B cell lymphomas	Complete, full results awaited	NCT01955668	-
	AD6738 ± chemotherapy	I	AZD6738 ± carboplatin, olaparib or MEDI4736	Solid tumors, HNSCC, ATM ^{loss} NSCLC, gastric or GOJ carcinoma	Recruiting	NCT02264678	-
		I	AZD6738 + paclitaxel	Solid tumors	Recruiting	NCT02630199	-
	AZD6738 + RT	I	AZD6738 + palliative RT	Solid tumors	Recruiting	NCT02223923	-
CHK1	MK8776 (SCH 900776)	II	Cytarabine ± MK8776 (randomised)	Relapsed AML	Complete, full results awaited	NCT01870596	-
		I	Gemcitabine + MK8776	Relapsed lymphoma	Complete, full results awaited	NCT00779584	(73)
		I	Cytarabine + MK8776	Relapsed AML	33% (8/24) CR	NCT00907517	(161)
	LY2603618	I / II	Cisplatin / pemetrexed ± LY2603618	NSCLC	14% (2/14) PR	NCT01139775	(162)
		I / II	Gemcitabine ± LY2603618	Pancreatic carcinoma	Complete, full results awaited	NCT00839332	-
		II	Pemetrexed + LY2603618	NSCLC	Complete, full results awaited	NCT00988858	-
		I	LY2603618 + pemetrexed or gemcitabine	Solid tumors	Complete, full results awaited	NCT01296568	-
		I	LY2603618 + gemcitabine	Solid tumors	Complete, full results awaited	NCT01341457	(74)
		I	LY2603618 + pemetrexed	Solid tumors	Complete, full results awaited	NCT00415636	-
	CCT245737	I	CCT245737	Solid tumors	Recruiting	NCT02797964	-
		I	CCT245737 + cisplatin and/or gemcitabine	Solid tumors	Recruiting	NCT02797977	-

	GDC-0575 ± chemotherapy	I	GDC-0575 ± gemcitabine	Solid tumors, relapsed lymphoma	Complete, full results awaited	NCT01564251	-
CHK1/2	LY2606368	II	LY2606368	Refractory SCLC	Recruiting	NCT02735980	-
		II	LY2606368	Ovarian, breast, prostate	Recruiting	NCT02203513	-
		II	LY2606368	Solid tumors	Recruiting	NCT02873975	-
		I	LY2606368	Solid tumors	4% (2/45) PR – anal SCC, HNSCC	NCT01115790	(77)
		I	LY2606368	Solid tumors	Recruiting	NCT02778126	-
		I	LY2606368	Solid tumors	Recruiting	NCT02514603	-
		I	LY2606368	Pediatric solid tumors	Recruiting	NCT02808650	-
	LY2606368 + chemotherapy	I	LY2606368 + fludarabine + cytarabine	Relapsed AML, high risk MDS	Recruiting	NCT02649764	-
		I	LY2606368 + cisplatin, cetuximab, pemetrexed, fluorouracil and/or leucovorin	Solid tumors	Recruiting	NCT02124148	-
	LY2606368 + targeted therapy	I	LY2606368 + ralimetinib (MAPK inhibitor)	Solid tumors	Recruiting	NCT02860780	-
	LY2606368 + RT	I	LY2606368 + RT + cisplatin or cetuximab	Locally advanced HNSCC	Recruiting	NCT02555644	-
WEE1	AZD1775	II	AZD1775	SCLC	Recruiting	NCT02593019	-
		I	AZD1775	Solid tumors	8% (2/25) PR: BRCA ^{mut}	NCT01748825	(78,83)

					ovarian, BRCA ^{mut} HNSCC		
		I	AZD1775	Solid tumors	Recruiting	NCT02482311	(163)
		I	AZD1775	Solid tumors	Recruiting	NCT02610075	-
	AZD1775 + chemotherapy	II	Carboplatin / paclitaxel ± AZD1775	Ovarian, TP53 ^{mut}	Superior PFS with AD1775 vs chemotherapy alone. Median PFS 42.8w vs 34.8w. HR 0.55 (95% CI 0.32 – 0.95, p= 0.03)	NCT01357161	(84)
		II	Carboplatin + AZD1775	Ovarian, TP53 ^{mut} or platinum resistant	27% (6/22) PR	NCT01164995	(85)
		II	Gemcitabine ± AZD1775	Ovarian, Primary Peritoneal, or Fallopian	Recruiting	NCT02101775	-
		II	Carboplatin / pemetrexed ± AZD1775	NSCLC, 1 st line	Complete, full results awaited	NCT02087241	-
		II	Docetaxel + AZD1775	NSCLC, 2 nd line	Closed	NCT02087176	-
		II	Carboplatin / Paclitaxel + AZD1775	NSCLC	Recruiting	NCT02513563	-
		II	Paclitaxel weekly + AZD1775	Gastric carcinoma	Recruiting	NCT02448329	-
		II	Cisplatin ± AZD1775	HNSCC	Complete, full results awaited	NCT02196168	-
		I / II	Gemcitabine / nab-paclitaxel ± AZD1775	Pancreatic	Recruiting	NCT02194829	-
		I / II	Irinotecan + AZD1775	Pediatric solid tumors	Recruiting	NCT02095132	-
		I	Cisplatin / docetaxel +	Locally advanced HNSCC	Recruiting	NCT02508246	-

			AZD1775				
		I	Gemcitabine, cisplatin or carboplatin + AZD1775	Solid tumors	Complete, full results awaited	NCT00648648	-
	AZD1775 + targeted therapy	I	AZD1775 + olaparib (PARP inhibitor)	Solid tumors	Complete, full results awaited	NCT02511795	(164)
			AZD1775 + Belinostat (HDAC inhibitor)	AML, other myeloid malignancies	Recruiting	NCT02381548	-
	AZD1775 + RT	I / II	Radiation + gemcitabine + AZD1775	Pancreatic	Recruiting	NCT02037230	-
		I	Radiation + cisplatin + AZD1775	HNSCC	Recruiting	NCT02585973	(165)
		I	RT + cisplatin + AZD1775	Locally advanced cervical cancer	Recruiting	NCT01958658	-
		I	Radiation + temozolomide + AZD1775	GBM	Recruiting	NCT01849146	-
		I	Radiation + AZD1775	Pediatric DIPG	Recruiting	NCT01922076	-
	AZD1775 + immune checkpoint inhibitor	I	MEDI4736 + AD1775	Solid tumors	Recruiting	NCT02617277	-
BER	TRC102	I	TRC102	Solid tumors, lymphoma	Complete, full results awaited	NCT01851369	(90)
	TRC102 + chemotherapy	II	TRC102 + temozolomide	GBM	Recruiting	NCT02395692	-

		I / II	TRC102 + cisplatin and/or pemetrexed	Solid tumors	Recruiting	NCT02535312	-
		I	TRC102 + pemetrexed	Solid tumors	4% (1/28) PR: HNSCC	NCT00692159	(89)
		I	TRC102 + fludarabine	Haematologic malignancies	Complete, full results awaited	NCT01658319	-
	TRC102 + RT	I	TRC + RT + cisplatin ± pemetrexed	NSCLC	Recruiting	NCT02535325	-

Table 2: Monotherapy and combination trials involving DDR inhibitors that have completed or are still active (www.clinicaltrials.gov). CLL, chronic lymphocytic leukemia; CR, complete response; CRUK, Cancer Research UK; DIPG, diffuse intrinsic pontine gliomas; ES, Ewing's sarcoma; GBM, glioblastoma multiforme; GOJ, gastro-oesophageal carcinoma; HDAC, Histone deacetylase; HGSOC, high grade serous ovarian carcinoma; HNSCC, head and neck squamous cell carcinoma; mAb, monoclonal antibody; NCI, National Cancer Institute; NSCLC, non-small cell lung carcinoma; ORR, objective response rate; PLD, pegylated liposomal doxorubicin; PLL, prolymphocytic leukaemia; PR, partial response; RT, radiotherapy; SCLC, small cell lung carcinoma. ¹ Dual DNA-PK and mTOR inhibitor.

FIGURE LEGENDS

Figure 1

Table showing predominant sensors, signaling and effector proteins for major DNA repair pathway. Main targets of drug development are in red (see text for details).

Figure 2

DNA DSB repair signaling pathways through the apical DDR kinases.

A. DNA-PK: Ku binds to DNA DSBs and recruits DNA-PKcs. Upon DNA binding, autophosphorylation of DNA-PKcs induces a conformational change that destabilises the NHEJ core complex, causing sliding of Ku inwards on the DNA and enabling access of end-processing and ligation enzymes to DNA ends and facilitation of repair.

B. ATM: Following DSBs ATM is predominantly activated through interactions with NBS1 of the MRN complex. ATM is the principle kinase responsible for phosphorylation of histone H2AX on serine 139 (known as γ H2AX). MDC1 (mediator of DNA damage checkpoint protein1) directly binds γ H2AX and potentiates DNA damage signaling leading to spreading of γ H2AX to over a megabase from its initial lesion. This in turn promotes recruitment and retention of DNA damage mediator proteins such as 53BP1. CHK2 is a well-studied ATM substrate.

C. ATR: ATR is activated by RPA (replication protein A) bound to ssDNA. The ATR-CHEK1 signaling cascade activates the G2-M checkpoint, promotes replication fork stabilisation and slows DNA replication by suppressing origin firing.